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(54) Title: CHIMERIC OPG POLYPEPTIDES			
(57) Abstract			
<p>Chimeric polypeptides comprising fusions of an osteoprotegerin dimerization domain to a heterologous sequence are provided. Also provided are nucleic acids encoding the polypeptides, expression vectors and host cells for their production and pharmaceutical compositions comprising the polypeptides.</p>			
<p>1 50 ThTdp/OPG NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI ThTdp 4.0 NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI ThTdp/196 NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI ThTdp/217 NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI ThTdp/248 NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI ThTdp/304 NLSSTVPLI LPLVLLRLV GIVPRVIGL VPHGDGEEI DSVCPQGYI</p> <p>51 100 ThTdp/OPG HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL ThTdp 4.0 HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL ThTdp/196 HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL ThTdp/217 HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL ThTdp/248 HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL ThTdp/304 HPQMSICTT KCKKOTYLN DCPQPGQDT CHERSGFT ASEDLNHL</p> <p>101 150 ThTdp/OPG SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL ThTdp 4.0 SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL ThTdp/196 SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL ThTdp/217 SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL ThTdp/248 SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL ThTdp/304 SCSCCKKED QVLSICTVD KDTVCCKRN QTHVWSEI PQCPHSLCL</p> <p>151 200 ThTdp/OPG KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE ThTdp 4.0 KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE ThTdp/196 KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE ThTdp/217 KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE ThTdp/248 KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE ThTdp/304 KQTVCTCHA QFLANECV SCSCCKKED CTCLCPQIE</p> <p>201 250 ThTdp/OPG NVKSTEDST TQKGLDVL CEAFPPFV PFTFPFMLE VLNDHJG ThTdp 4.0 NVKSTEDST T...GLDVL CEAFPPFV PFTFPFMLE VLNDHJG ThTdp/196 NVKSTEDST T...GLDVL CEAFPPFV PFTFPFMLE VLNDHJG ThTdp/217 NVKSTEDST TQ...GLDVL CEAFPPFV PFTFPFMLE VLNDHJG ThTdp/248 NVKSTEDST TQ...GLDVL CEAFPPFV PFTFPFMLE VLNDHJG ThTdp/304 NVKSTEDST TQ...GLDVL CEAFPPFV PFTFPFMLE VLNDHJG</p> <p>251 300 ThTdp/OPG VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF ThTdp 4.0 VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF ThTdp/196 VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF ThTdp/217 VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF ThTdp/248 VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF ThTdp/304 VNAESVERIK RQNSRQDT QLLKLNKHQ KDDIVKAI QDIDLCDF</p> <p>301 350 ThTdp/OPG QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL ThTdp 4.0 QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL ThTdp/196 QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL ThTdp/217 QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL ThTdp/248 QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL ThTdp/304 QRTGHANLT FQGLSLAES LPOKVAED IERTTRACP EQDILKSL</p> <p>351 400 ThTdp/OPG WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY ThTdp 4.0 WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY ThTdp/196 WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY ThTdp/217 WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY ThTdp/248 WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY ThTdp/304 WRKNDQDT LKMLHALH SKTYHPTV TQSLNTIF LSPFHYLY</p> <p>401 450 ThTdp/OPG QLFLELIGH QGVSVKSL ThTdp 4.0 QLFLELIGH QGVSVKSL ThTdp/196 QLFLELIGH QGVSVKSL ThTdp/217 QLFLELIGH QGVSVKSL ThTdp/248 QLFLELIGH QGVSVKSL ThTdp/304 QLFLELIGH QGVSVKSL</p> <p>401 (OPG)</p>			

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CHIMERIC OPG POLYPEPTIDES

Field of the Invention

5 The invention relates generally to chimeric polypeptides. More particularly, the invention relates to chimeric polypeptides comprising a fusion of an osteoprotegerin dimerization domain to a heterologous sequence. The polypeptides may be used in a variety of
10 diagnostic and therapeutic applications.

Background of the Invention

 Cells recognize a variety of signals which
15 modulate growth, differentiation and metabolism. Effectors of cellular functions include small molecular weight organic compounds, carbohydrates, amino acids, peptides and proteins. At present, the best understood signalling process employs secretion of a signalling
20 molecule from one cell to modulate functions of other cells (autocrine regulation). It has also been observed that secreted signalling molecules may also modulate the functions of cells which secrete them (paracrine regulation). The ability of cells to
25 respond to external signals usually requires that the appropriate receptors which bind the signalling molecules be present on the cell surface. Protein-mediated signalling between cells involves binding of growth factors, hormones, cytokines, cell adhesion
30 proteins and the like to cell surface receptors.

 As a class of proteins, receptors vary in their structure and mode of signal transduction. They are characterized by having an extracellular domain that is involved in binding a signalling molecule and

cytoplasmic domain which transmits an appropriate intracellular signal. Receptor expression patterns ultimately determine which cells will respond to a given ligand, while the structure of a given receptor dictates the cellular response induced by ligand binding. Receptors have been shown to transmit intracellular signals via their cytoplasmic domains by activating protein tyrosine, or protein serine/threonine phosphorylation (e.g., platelet derived growth factor receptor (PDGFR) or transforming growth factor- β receptor-I (TGF β R-I), by stimulating G-protein activation (e.g., β -adrenergic receptor), and by modulating associations with cytoplasmic signal transducing proteins (e.g., TNFR-1 and Fas/APO) (Heldin, Cell 80, 213-223 (1995)).

The tumor necrosis factor receptor (TNFR) superfamily is a group of type I transmembrane proteins which share a conserved cysteine-rich motif which is repeated three to six times in the extracellular domain (Smith, et al. Cell 76, 953-962 (1994)). Collectively, these repeat units form the ligand binding domains of these receptors (Chen et al., Chemistry 270, 2874-2878 (1995)). The ligands for these receptors are a structurally related group of proteins homologous to TNF α . (Goeddel et al. Cold Spring Harbor Symp. Quart. Biol. 51, 597-609 (1986); Nagata et al. Science 267, 1449-1456 (1995)). TNF α binds to distinct, but closely related receptors, TNFR-1 and TNFR-2. TNF α produces a variety of biological responses in receptor bearing cells, including, proliferation, differentiation, and cytotoxicity and apoptosis (Beutler et al. Ann. Rev. Biochem. 57, 505-518 (1988)).

TNF α is believed to mediate acute and chronic inflammatory responses (Beutler et al. ibid). Systemic delivery of TNF α induces septic shock-like syndrome and

widespread tissue necrosis. Because of this, TNF α may be responsible for the severe morbidity and mortality associated with a variety of infectious diseases, including sepsis. Mutations in FasL, the ligand for the TNFR-related receptor Fas/APO (Suda et al. Cell 75, 1169-1178 (1993)), is associated with autoimmunity (Fisher et al. Cell 81, 935-946 (1995)), while overproduction of FasL may be implicated in drug-induced hepatitis. Thus, ligands to the various TNFR-related proteins often mediate the serious effects of many disease states, which suggests that agents that neutralize the activity of these ligands would have therapeutic value.

Soluble TNFR-1 receptors and antibodies that bind TNF α have been tested for their ability to neutralize systemic TNF α (Loetscher et al. Cancer Cells 3, 221-226 (1991)). A naturally occurring form of a secreted TNFR-1 and TNFR-2 mRNA was recently cloned, and its product tested for its ability to neutralize TNF α activity in vitro and in vivo (Kohn et al. Proc. Natl. Acad. Sci. USA 87, 8331-8335 (1990)). The ability of this protein to neutralize TNF α suggests that soluble TNF receptors function to bind and clear TNF thereby blocking the cytotoxic effects on TNFR-bearing cells.

Recombinantly-produced TNF inhibitors have also been taught in the art. For example, EP 393 438 and EP 422 339 teach the amino acid and nucleic acid sequences of a "30kDa TNF inhibitor" (also known as a p55 receptor) and a "40kDa inhibitor" (also known as a p75 receptor) as well as modified forms thereof, e.g., fragments, functional derivatives and variants. EP 393 438 and EP 422 339 also disclose methods for isolating the genes responsible for coding the

inhibitors, cloning the gene in suitable vectors and cell types, and expressing the gene to produce the inhibitors. Mature recombinant 30kDa TNF inhibitor and mature recombinant 40kDa TNF inhibitor have
5 been demonstrated to be capable of inhibiting TNF (EP 393 438, EP 422 339, PCT Publication No. WO 92/16221 and PCT Publication No. WO 95/34326).

A recently identified member of the TNFR family, termed Osteoprotegerin (OPG), is a secreted
10 polypeptide which inhibits osteoclast maturation and markedly increases bone density in transgenic mice expressing the OPG polypeptide. OPG inhibited in vitro the formation of mature osteoclasts from hematopoietic progenitor cells and reduced the extent of bone loss in
15 ovariectomized rats (see co-owned and co-pending U.S. Serial Nos. 08/577,788, filed December 22, 1995; 08/706,945, filed September 3, 1996; and 08/771,777 filed December 20, 1996). OPG may have benefit in the treatment of osteopenia. PCT Application No.
20 WO96/26217 discloses a polypeptide termed Osteoclastogenesis Inhibitory Factor (OCIF) which is identical to OPG.

OPG comprises two domains having different structural and functional properties. The
25 amino-terminal domain spanning residues 22-194 in the mature polypeptide shows homology to other members of the TNFR family, especially TNFR-2, through conservation of cysteine rich domains characteristic of TNFR family members. The carboxy terminal domain
30 spanning residues 194-401 has no significant homology to any known sequences. Unlike a number of other TNFR family members, OPG appears to be exclusively a secreted protein and does not appear to be synthesized as a membrane associated form. Analysis of OPG by
35 reducing and non-reducing gel electrophoresis indicated that the full-length mature polypeptide of 380 amino

acids formed a dimer having a molecular weight of about 120 kDa as compared to the monomer molecular weight of about 60 kDa. OPG polypeptides having certain truncations in the carboxy terminal domain or
5 substitutions of certain cysteine residues within in the carboxy terminal domain formed dimeric OPG to a lesser extent and had lower biological activity compared to wild-type OPG. However, replacement of part or all of the OPG carboxy terminal domain with an
10 Fc region of IgG restored biological activity in the OPG fusion protein to near normal levels. Based upon these observations, the amino-terminal region of OPG appeared to be required for biological activity while the carboxy-terminal domain was important for
15 dimerization. In addition, the biological activity of OPG appeared to be enhanced when the molecule was in dimeric form.

In a therapeutic regimen, it is often desirable to modulate a biological response either by
20 enhancing or blocking a signal received by a receptor. Enhancement of a biological response can involve increasing the affinity of the signalling molecule for a receptor, or increasing the half-life of the molecule in circulation such that it is bound to the receptor
25 for a longer period of time. When the signalling molecule is a polypeptide, enhancement of a biological response may be achieved by constructing analogs which have amino acid sequence changes that increase binding or half-life, derivatives (e.g., polypeptides modified
30 with water soluble polymers) to increase solubility and/or half-life, or chimeric polypeptides (e.g, polypeptides fused to the Fc region of IgG) which increase half-life, solubility and/or modify the aggregation state of the protein in circulation.
35 Similar approaches may be taken to develop therapeutic proteins which act as antagonists by blocking a

biological response. In particular, soluble forms of transmembrane receptors which may encompass part or all of the extracellular domains have been used to prevent ligand binding and receptor activation. Soluble
5 receptors have been developed as chemically-modified derivatives and as chimeric polypeptides.

Due to the relatively low inhibition of cytotoxicity exhibited by the 30kDa TNF inhibitor and 40kDa TNF inhibitor (Butler et al. Cytokine 6, 616-623
10 (1994)), various groups have generated dimers of TNF inhibitor proteins (Butler et al. (1994), supra; and Martin et al. Exp. Neurol. 131, 221-228 (1995)). However, the dimers may generate an antibody response (Martin et al. (1995), supra; and Fisher et al. New
15 Eng. J. Med., 334, 1697-1702 (1996)).

Generation of chimeric polypeptides has been described in the art. For example, construction of hybrid immunoglobulin molecules by fusion of a ligand binding partner to a human IgG chain is described in
20 U.S. Patent Nos. 5,116,964 and 5,428,130. Construction of a chimeric polypeptide comprising the extracellular domain of a TNF receptor fused to a mouse IgG heavy chain is described in U.S. Patent No. 5,447,851. Chimeric polypeptides comprising the
25 extracellular domain of a human PDGF receptor fused to dimerizing proteins is described in EP 0 721 983. Multimers of soluble forms of TNF receptors are described in U.S. Patent No. 5,478,925.

While fusion proteins, such as those
30 comprising immunoglobulin constant regions, may have desirable biological properties, they can elicit an immune response which limits their usefulness as a human therapeutic.

Therefore, it is an object of the invention
35 to provide chimeric polypeptides which enhance or block a biological response. Such polypeptides may have

increased stability, solubility, circulating half-life and decreased immunogenicity.

It is another object of the invention to provide chimeric polypeptides which combine the active
5 region of a signalling molecule with an OPG dimerization domain wherein said chimeric polypeptides will enhance or block a biological response characteristic of the signalling molecule portion of the chimera.

10 It is another object of the invention to provide OPG chimeric polypeptides which form dimers, trimers and higher multimers which may have advantageous properties such as increased binding affinity, greater stability, and longer circulating
15 half-life compared to monomeric forms.

Summary of the Invention

The invention provides for chimeric polypeptides comprising fusions of an OPG dimerization
20 domain to a heterologous sequence. Also provided for are nucleic acid sequences encoding the polypeptides, expression vectors and host cells for production of the polypeptides, and pharmaceutical compositions comprising the polypeptides.

25 A heterologous sequence of the invention comprises an amino acid sequence of a cell signalling molecule, such as a receptor, an extracellular domain thereof, and an active fragment, derivative and analog of a receptor or an extracellular domain. In a
30 preferred embodiment, heterologous sequences are selected from the family of TNF-like receptors. Such sequences preferentially include functional extracellular ligand binding domains and lack functional transmembrane and cytoplasmic domains. In
35 another embodiment, the transmembrane and cytoplasmic domains are deleted in whole or in part. It is

understood that heterologous sequences of the invention do not include the amino terminal region of OPG defined by residues 22-194 as shown in U.S. Serial No.

08/577,788 filed December 22, 1995 and hereby

- 5 incorporated by reference, and do not include related amino acid sequences which, when fused to an OPG dimerization domain, exhibit the biological activity of OPG.

- Also encompassed by the invention are
- 10 multimeric polypeptides comprising covalently associated monomers of OPG chimeric polypeptides. The monomers may have identical heterologous sequences or different heterologous sequences. In a preferred embodiment, the multimeric polypeptide is a dimer,
- 15 either a heterodimer (different heterologous sequences) or a homodimer (identical heterologous sequences).

- The chimeric polypeptides of the invention are produced by transforming or transfecting host cells with nucleic acids encoding the polypeptide, culturing
- 20 the host cells, and recovering the polypeptide from the culture. Also provided for are expression vectors and host cells for producing the chimeric polypeptides.

- The chimeras are useful for detecting molecules which interact with fused heterologous
- 25 sequences and thereby identifying potential new receptors and ligands. The compositions of chimeric polypeptides provided herein are useful for treatment of a variety of disorders, for example those related to receptor binding. In one embodiment, compositions
- 30 comprising TNF/OPG and TNFR/OPG chimeric are used to treat TNF and TNFR mediated disorders, such as inflammation, autoimmune diseases, and disorders related to excessive apoptosis

Description of the Figures

Figure 1: Amino acid sequences of human, mouse and rat OPG dimerization domains (residues 194-401 of corresponding full-length OPG polypeptides). Conserved cysteine residues implicated in disulfide bond formation are underlined.

Figure 2. Nucleic acid and amino acid sequence of mature, full-length 30 kDa TNF inhibitor.

Figure 3. Nucleic acid and amino acid sequence of mature, full-length 40 kDa TNF inhibitor.

Figure 4. Amino acid sequences of TNFbp/OPG chimeric polypeptides. The TNFbp portion of the chimera is the full-length 30 kDa TNF inhibitor with the leader sequence (underlined) and the additional sequence VKGTEDSGTT at the carboxy terminus. OPG dimerization domains are human OPG residues 194-401, 196-401, 217-401, 248-401 and 304-401. The junction of the TNFbp and OPG sequences creates an Age I restriction site in the DNA sequence and adds a glycine codon (at position 212).

Figure 5. Gel electrophoresis analysis of TNFbp/OPG chimeric polypeptides. TNFbp/OPG chimeric plasmids were transfected into CHO d-cells. supernatants from serum-free roller bottle harvests were analyzed on a 12% polyacrylamide, Tris-glycine, non-reducing gel. Dimerization patterns were compared to a TNFbp-Fc fusion (lane 1) and TNFbp monomer (lane 8).

Figure 6. Inhibition of TNF α cytotoxicity on L929 cells. Serum-free conditioned medium samples of TNFbp/Fc and TNFbp/OPG [194-401] fusion polypeptides were serially diluted and assayed for inhibition of TNF α cytotoxicity on L929 cells.

Detailed Description of the Invention

The invention provides for a chimeric polypeptide comprising a fusion of an OPG dimerization domain to a heterologous sequence.

The term "heterologous sequence" refers to an amino acid sequence which is involved in cell signalling and acts to modulate cell growth, differentiation or metabolism. In general, heterologous sequences comprise extracellular ligand binding domains of cell surface receptors and their cognate ligands. When present as part of an OPG chimeric polypeptide, a heterologous sequence of the invention comprises about ten or more amino acids in length, about 20 or more amino acids in length, about 50 or more amino acids in length, and about 100 or more amino acids in length. A heterologous sequence will be of sufficient size to confer on a chimeric polypeptide a functional property such as receptor binding, enzymatic activity, inhibitor activity and the like; however, it is understood that the chimeric polypeptides will not have functional properties identical to OPG although they may share one or more functions in common with OPG. Heterologous sequences may encode full-length polypeptides or active fragments, derivatives and analogs thereof.

In preferred embodiments, chimeric OPG polypeptides include heterologous sequences encoding growth factors, cytokines, hormones, cell adhesion molecules and other polypeptide factors which are

typically secreted. Chimeric OPG polypeptides also include heterologous sequences which encode receptors for growth factors, cytokines, hormones, cell adhesion molecules, and the like, and preferably will include
5 extracellular ligand binding domains from said receptors, and active fragments, derivatives and analogs thereof. The heterologous sequences may or may not be capable of forming dimers or higher aggregates when the sequences are present in a naturally occurring
10 form.

The "OPG dimerization domain" refers to that portion of the OPG polypeptide which is capable of forming covalently associated multimeric polypeptides. It is understood, however, that chimeric polypeptides
15 comprising an OPG dimerization domain are not restricted to forming dimers, but may form higher multimers as well (trimers, tetramers, etc.) The domain may have the amino acid sequence of the human osteoprotegerin dimerization domain, or it may be a
20 fragment, derivative or analog thereof which is capable of forming covalently associated multimers. More specifically, an OPG dimerization domain will retain one or more cysteine residues which will allow formation of at least one interchain disulfide bond.
25 In a preferred embodiment, the OPG dimerization domain has the amino acid sequence from about residues 194 to 401 inclusive of human OPG.

As used herein, the term "fragment" comprises a deletion of one or more amino acids in a heterologous
30 sequence or in an OPG dimerization domain. The deletion may occur at the amino terminal end, the carboxy terminal end or in an internal region of the sequence. As used herein, the term "derivative" refers to a modification of the polypeptide backbone of an OPG
35 chimera, either within the OPG dimerization domain or within the heterologous sequence. Said modifications

include, but are not limited to, attachment of water soluble polymers, hydrophobic moieties, fluorescent tags, enzymatic labels and the like. As used herein, the term "analogs" refers to one or more amino acid
5 substitutions and/or insertions within a polypeptide. Substitutions may involve conservative replacements or non-conservative replacements of amino acids which are known to one skilled in the art. Amino acid insertions may occur at the amino or carboxy terminal ends of
10 either the OPG dimerization domain or the heterologous sequence or both, or may occur in internal regions.

Polypeptides

Chimeric polypeptides of the invention
15 comprise a heterologous sequence fused at its carboxy terminus to the amino terminus an OPG dimerization domain or, alternatively, an OPG dimerization domain fused at its carboxy terminus to the amino terminus of a heterologous sequence. Chimeric polypeptides may be
20 constructed as a direct fusion of a heterologous sequence and an OPG dimerization domain or may be constructed with a spacer or adapter region having one or more amino acids inserted between the two portions of the polypeptide. Optionally, the spacer region may
25 encode a protease cleavage site. The precise site of the fusion is not critical and may be varied by one skilled in the art in order to optimize binding characteristics and/or biological activity of the heterologous sequence.

30 According to the invention, an OPG dimerization domain may be mammalian in origin (such as from mouse, rat or human) or may be a fragment or analog thereof which is capable of forming covalently associated dimers or higher order multimers. The amino
35 acid sequences of rat, mouse and human OPG dimerization domains span from about residues 194-401 of their

respective full-length OPG polypeptides as shown in Figure 1 (SEQ ID NO:___). Fragments and analogs of an OPG dimerization domain include: deletion or substitution of a cysteine residue at any of positions 195, 202, 277, 319 and 400; addition of one or more cysteine residues; rearrangement of the configuration of cysteine residues which may entail a net increase from, a net decrease from, or no change in the number of cysteine residues compared to residues 194-401 of the human OPG dimerization domain; amino-terminal truncations of OPG[194-401], e.g, 195-401, 196-401, and so forth; C-terminal truncations of OPG[194-401], e.g, 194-400, 194-399, and so forth; conservative substitutions of amino acid residues in OPG[194-401] wherein the substitutions comprise replacements with structurally or functionally similar amino acids which are known to one skilled in the art; and any combinations thereof.

Heterologous sequences which form part of a chimeric OPG polypeptide include receptors having known extracellular ligand binding domains. Examples are receptor protein-tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR) family, fibroblast growth factor receptor (FGFR) family, insulin receptor family, epidermal growth factor receptor (EGFR) family, nerve growth factor (NGFR) family, hepatocyte growth factor family (HGFR), EPH family, AXL family, TIE family, DDR family, ROR family, and other receptor protein tyrosine kinases (see van der Geer et al. Ann. Rev. Cell Biol. 10, 251-337 (1994)). Other examples of receptors having extracellular ligand binding domains include the cytokine receptor superfamily, such as G-CSF, GM-CSF (α and β subunits), MGF, EPO, MGDF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, growth hormone, α -

- interferon, β -interferon, and γ -interferon receptors, the seven transmembrane domain receptor superfamily, such as acetylcholine, adrenergic, dopamine, thrombin, FSH, gonadotropin, thyrotropin, clacitonin and
- 5 parathyroid hormone receptors, and cell adhesion receptors. It is understood that the receptors cited herein are merely examples and that heterologous sequences present in OPG chimeric polypeptides are not limited to the above-mentioned receptors.
- 10 Other heterologous sequences of the invention comprise growth factors, hormones, cytokines, cell adhesion proteins and the like. Also included are corresponding ligands for the receptor protein tyrosine kinases, ligands for cytokine receptors, ligands for
- 15 seven transmembrane domain receptors, and ligands for cell adhesion receptors.
- In a preferred embodiment, the heterologous sequence is a member of the TNF receptor superfamily or is derived from a member of the TNF receptor family.
- 20 Members include TNFR-1, TNFR-2, TNFRp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB. Typically the extracellular domains of TNF receptors, or active fragments, derivatives and analogs thereof, are fused to an OPG dimerization domain. Active fragments of TNF
- 25 receptors will have at least one cysteine rich domain, alternatively two, three or four cysteine rich domains, or alternatively one, two or three cysteine rich domains and a portion thereof, for example, two cysteine rich domains and a portion of a third domain.
- 30 Activity of a TNF/OPG chimeric polypeptide may include biological activity or ligand binding activity characteristic of a TNF family member which may be evaluated using procedures known to one skilled in the art.
- 35 Preferred heterologous sequences comprise TNFR-1 or are derived from TNFR-1, and may be

a 30kDa TNF inhibitor, a 40 kDa TNF inhibitor, or a functionally active low molecular weight TNF inhibitor. The nucleic acid and amino acid sequence of mature, full-length 30kDa TNF inhibitor is shown in Figure 2 (SEQ ID NO:___). The nucleic acid and amino acid sequence of mature, full-length 40kDa TNF inhibitor is shown in Figure 3 (SEQ ID NO:___). The low molecular weight TNF inhibitors are modified forms of the 30kDa TNF inhibitor and 40 kDa TNF inhibitor which do not contain the fourth domain (amino acid residues Thr¹²⁷-Thr¹⁶¹ of the 30kDa TNF inhibitor and amino acid residues Pro¹⁴¹-Thr¹⁷⁹ of the 40kDa TNF inhibitor); a portion of the third domain (amino acid residues Asn¹¹¹-Cys¹²⁶ of the 30kDa TNF inhibitor and amino acid residues Pro¹²³-Lys¹⁴⁰ of the 40kDa TNF inhibitor); and, optionally, which do not contain a portion of the first domain (amino acid residues Asp¹-Lys²¹ of the 30kDa TNF inhibitor and amino acid residues Leu¹-Lys³⁴ of the 40kDa TNF inhibitor).

The heterologous sequences of the present invention include derivatives of TNFR-1 proteins represented by the formula R₁-[Cys¹⁹-Cys¹⁰³]-R₂ and R₄-[Cys³²-Cys¹¹²]-R₅. These proteins are deletion variants of the 30kDa TNF inhibitor and the 40kDa TNF inhibitor, respectively, and are referred to as "truncated TNFbp(s)".

By "R₁-[Cys¹⁹-Cys¹⁰³]-R₂" is meant one or more proteins wherein [Cys¹⁹-Cys¹⁰³] represents residues 19 through 103 of mature, full-length 30kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 2 (SEQ ID NO:___) to facilitate the comparison; wherein R₁ represents a methionylated or nonmethionylated amine group of Cys¹⁹ or of amino-terminus amino acid residue(s) selected from the group:

16

C
 IC
 SIC
 NSIC (SEQ ID NO:___)
 NNSIC (SEQ ID NO:___)
 QNNSIC (SEQ ID NO:___)
 PQNNSIC (SEQ ID NO:___)
 HPQNNSIC (SEQ ID NO:___)
 IHPQNNSIC (SEQ ID NO:___)
 YIHPQNNSIC (SEQ ID NO:___)
 KYIHPQNNSIC (SEQ ID NO:___)
 GKYIHPQNNSIC (SEQ ID NO:___)
 QGKYIHPQNNSIC (SEQ ID NO:___)
 PQGKYIHPQNNSIC (SEQ ID NO:___)
 CPQGKYIHPQNNSIC (SEQ ID NO:___)
 VCPQGKYIHPQNNSIC (SEQ ID NO:___)
 SVCPQGKYIHPQNNSIC (SEQ ID NO:___)
 DSVCPQGKYIHPQNNSIC (SEQ ID NO:___);

and wherein R₂ represents a carboxy group of Cys¹⁰³ or
 of carboxy-terminal amino acid residues selected from
 5 the group:

F
 FC
 FCC
 FCCS (SEQ ID NO:___)
 FCCSL (SEQ ID NO:___)
 FCCSLC (SEQ ID NO:___)
 FCCSLCL (SEQ ID NO:___);

and variants thereof.

Exemplary tumor necrosis factor binding
 10 proteins which comprise TNFbp/OPG chimeric polypeptides

of the present invention include the following molecules: NH₂-MDSVCPQGKYIHPQNNSIC-[Cys¹⁹-Cys¹⁰³]-FC-COOH (also referred to as 30kDa TNFbp 2.6C105); NH₂-MDSVCPQGKYIHPQNNSIC-[Cys¹⁹-Cys¹⁰³]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6C106); NH₂-MDSVCPQGKYIHPQNNSIC-[Cys¹⁹-Cys¹⁰³]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.6N105); NH₂-MYIHPQNNSIC-[Cys¹⁹-Cys¹⁰³]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d8); NH₂-M-[Cys¹⁹-Cys¹⁰³]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d18); and NH₂-MSIS-[Cys¹⁹-Cys¹⁰³]-FNCSL-COOH (also referred to as 30kDa TNFbp 2.3d15), either methionylated or nonmethionylated, and variants and derivatives thereof.

By "R₄-[Cys³²-Cys¹¹²]-R₅" is meant one or more proteins wherein [Cys³²-Cys¹¹²] represents residues Cys³² through Cys¹¹² of mature, full-length 40kDa TNF inhibitor, the amino acid residue numbering scheme of which is provided in Figure 3 (SEQ ID NO:__) to facilitate the comparison; wherein R₄ represents a methionylated or nonmethionylated amine group of Cys³² or of amino-terminus amino acid residue(s) selected from the group:

C	
MC	
QMC	
AQMC	(SEQ ID NO:__)
TAQMC	(SEQ ID NO:__)
QTAQMC	(SEQ ID NO:__)
DQTAQMC	(SEQ ID NO:__)
YDQTAQMC	(SEQ ID NO:__)
YYDQTAQMC	(SEQ ID NO:__)
EYYDQTAQMC	(SEQ ID NO:__)
REYYDQTAQMC	(SEQ ID NO:__)
LREYYDQTAQMC	(SEQ ID NO:__)

RLREYYDQTAQMC	(SEQ ID NO:___)
CRLREYYDQTAQMC	(SEQ ID NO:___)
TCRLREYYDQTAQMC	(SEQ ID NO:___)
STCRLREYYDQTAQMC	(SEQ ID NO:___)
GSTCRLREYYDQTAQMC	(SEQ ID NO:___)
PGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
EPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
PEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
APEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
YAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
PYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
TPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
FTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
AFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
VAFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
QVAFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
AQVAFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
PAQVAFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___)
LPAQVAFTPYAPEPGSTCRLREYYDQTAQMC	(SEQ ID NO:___);

and wherein R₅ represents a carboxy group of Cys¹¹² or of carboxy-terminal amino acid residues selected from the group:

5

R	
RL	
RLC	
RLCA	(SEQ ID NO:___)
RLCAP	(SEQ ID NO:___)
RLCAPL	(SEQ ID NO:___)
RLCAPLR	(SEQ ID NO:___)
RLCAPLRK	(SEQ ID NO:___)
RLCAPLRKC	(SEQ ID NO:___)
RLCAPLRKCR	(SEQ ID NO:___)

and variants thereof.

As shown in Example 1, a hybrid DNA molecule encoding TNFbp 4.0, the full-length 30 kDa TNF inhibitor (Figure 2) with the additional sequence VKGTEDSGTT extending from the carboxy terminus, and human OPG [194-401] was constructed. The resulting chimeric polypeptide, termed TNFbp/OPG[194-401] has the amino acid sequence as shown in Figure 4. Upon expression, the mature chimeric polypeptides formed dimers in conditioned medium of transfected host cells as determined by non-reducing SDS-PAGE (see Figure 5). Additional TNFbp fusions were constructed to amino terminal truncations of the human OPG dimerization domain. These constructs are designated TNFbp/OPG[196-401], TNFbp/OPG[217-401], TNFbp/OPG[248-401], and TNFbp/OPG[304-401] and the amino acid sequences are shown in Figure 4. OPG[194-401] has the full complement of five cysteine residues which are involved in covalent association of OPG dimerization domains. OPG[196-401] lacks one cysteine residue at position 195, OPG[217-401] and OPG[248-401] lacks a second cysteine residue at position 202, and OPG[304-401] lacks a third cysteine residue at position 277 (see Figure 1 for location of cysteine residues). The chimeric polypeptides produced in conditioned medium of transfected CHO^d- host cells were analyzed by non-reducing SDS-PAGE (Figure 5). In the L929 cytotoxicity assay, the TNFbp/OPG[194-401] chimera showed activity similar to a TNFbp/Fc chimera (Figure 6).

The invention also provides for chimeric OPG polypeptides which form multimers (i.e., dimers, trimers and higher multimers). Multimers of the invention comprise covalently associated monomeric OPG chimeras wherein the monomers may have identical heterologous sequence or different heterologous sequences. Preferably, the chimeric polypeptides are

dimers or trimers. Preparations of multimeric polypeptides will be essentially free of monomeric OPG chimeras which are not covalently associated and of inactive multimers. Such preparations are made using techniques available to one skilled in the art

Modifications of chimeric OPG polypeptides are encompassed by the invention and include post-translational modifications (e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of OPG which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1kDa and about 100kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules

will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release
5 desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

10 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the
15 art, e.g. EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al. Exp. Hematol. 20, 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through
20 amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the
25 N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol
30 molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire N-terminally chemically modified protein. Using polyethylene
35 glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol

molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemically modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The chimeric OPG polypeptides of the invention are isolated and purified from other constituents present in lysates or supernatants of host cells expressing the polypeptides. In one embodiment, the polypeptide is free from association with other human proteins, such as the expression product of a bacterial host cell. Also provided by the invention is a method for the purification of OPG chimeric polypeptides. The purification process may employ one or more standard protein purification steps in an appropriate order to obtain purified protein. The chromatography steps can include ion exchange, gel filtration, hydrophobic interaction, reverse phase, chromatofocusing, affinity chromatography employing an anti-OPG antibody or biotin-streptavidin affinity complex and the like. When preparations of selected multimeric OPG chimeras are desired, the purification

method may be carried out to separate species of different aggregation states, for example, separation of monomeric from dimeric OPG chimeras, or separation of dimeric from tetrameric OPG chimeras.

5 Chimeric OPG polypeptides may be used in assays to screen for binding molecules. Examples of such molecules include, but are not limited to, nucleic acids, polypeptides, small molecular weight peptides, carbohydrates, lipids and small molecular weight
10 organic compounds. Assays will employ combining candidate molecules (either purified or unpurified) with chimeric OPG polypeptides under conditions that allowing binding, and measuring the extent of binding to the chimeric polypeptide. Binding measurements are
15 made using detection systems available to one skilled in the art, such as radioactivity, enzymatic activity, fluorescence, and surface plasmon resonance.

Nucleic Acids

20 The invention provides for an isolated nucleic acid encoding a chimeric polypeptide having an OPG dimerization domain fused to a heterologous sequence. The nucleic acids encode a chimeric OPG polypeptide wherein the heterologous sequence is a cell
25 signalling molecule such as a receptor or a receptor ligand. In a preferred embodiment, the heterologous nucleic acid sequence encodes a polypeptide of the TNFR family, or a fragment, derivative or analog thereof, provided however that the heterologous nucleic acid
30 sequence does not encode OPG[22-194] as shown in U.S. Serial No. 08/577,788 filed December 22, 1995, or a homologous sequence which, when fused to an OPG dimerization domain, has the biological activity of OPG.

35 The nucleic acids of the invention encode chimeric OPG polypeptides selected from the following:

a) the nucleic acid sequences which encode the polypeptides shown in Figure 1 (SEQ ID NO: ____) or complementary strands thereof; and

5 b) the nucleic acids sequences which hybridize under high stringency conditions with the sequences in (a), and degenerate sequences thereof, provided however that the polypeptides do not have the biological activity of OPG. Nucleic acids encoding OPG chimeric polypeptides may hybridize over part or all of
10 the nucleic acid sequences encoding the OPG dimerization domains shown in Figure 1 (SEQ ID NO: ____).

The conditions for hybridization are generally of high stringency using temperatures,
15 solvents and salt concentrations wherein the hybridizing sequences are about 12-20°C below the melting temperature (T_m) of the perfectly matched duplex. Equivalent stringency to these conditions may be readily ascertained by one skilled in the art by
20 adjusting salt and organic solvent concentrations and temperature. Specific hybridization conditions are described in Sambrook et al. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)

25 Preferred sequences include nucleic acids which encode chimeric OPG polypeptides having rat, mouse and human OPG dimerization domains. DNA encoding human OPG dimerization domain was provided in a full-length human OPG plasmid designated pRcCMV - human OPG
30 and deposited with the American Type Culture Collection, Rockville, MD on December 27, 1995 under accession no. 69969. DNA encoding rat OPG dimerization domain was provided in a full-length rat OPG plasmid designated pMOB-B1.1 and deposited with the American
35 Type Culture Collection, Rockville, MD on December 27,

1995 under ATCC accession no. 69970. DNA encoding mouse OPG dimerization domain was provided in a full-length mouse OPG plasmid designated pRcCMV-murine OPG and deposited with the American Type Culture

5 Collection, Rockville, MD on December 27, 1995 under accession no. 69971. The nucleic acids of the invention will hybridize under stringent conditions to the DNA inserts of ATCC accession nos. 69969, 69970, and 69971.

10 In a preferred embodiment, heterologous sequences will comprise nucleic acids encoding TNFR-1, and fragments, derivatives and analogs thereof, such as the TNF 30kDa inhibitor or TNF 40kDa inhibitor. Presently preferred heterologous sequences include
15 those nucleic acids encoding 30kDa TNFbp 2.6C105, 30kDa TNFbp 2.6C106, 30kDa TNFbp 2.6N105, 30kDa TNFbp 2.3d8, 30kDa TNFbp 2.3d18 and 30kDa TNFbp 2.3d15.

Also provided by the invention are nucleic acids encoding variants of an OPG chimeric polypeptide
20 wherein the variations may be in the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives comprise addition, substitution, insertion or deletion of one or more nucleotides such that the resulting sequences encode
25 chimeric OPG polypeptides comprising one or more amino acid residues which have been added, deleted, inserted or substituted in either the heterologous sequence or the OPG dimerization domain or both. The nucleic acid derivatives may be naturally occurring, such as by
30 splice variation or polymorphism, or may be constructed using site-directed mutagenesis techniques available to the skilled worker. Chimeric OPG polypeptide variants are described in the previous section entitled
"Polypeptides" and it is anticipated that nucleic acids
35 encoding all variants disclosed therein, and degenerate molecules thereof, are encompassed by the invention.

Examples of the nucleic acids of the invention include cDNA, genomic DNA, synthetic DNA and RNA. cDNA is obtained from libraries prepared from mRNA isolated from various tissues expressing OPG. In humans, tissue sources for OPG include kidney, liver, placenta and heart. Genomic DNA encoding OPG is obtained from genomic libraries which are commercially available from a variety of species. Synthetic DNA is obtained by chemical synthesis of overlapping oligonucleotide fragments followed by assembly of the fragments to reconstitute part or all of the coding region and flanking sequences (see U.S. Patent No. 4,695,623). RNA may be obtained in large quantities use of procaryotic expression vectors which direct high-level synthesis of mRNA, such as vectors using T7 promoters and RNA polymerase.

Nucleic acid sequences of the invention are useful for the expression of chimeric OPG polypeptides. Expression may be carried out in transfected host cells for production of recombinant protein in quantities sufficient for diagnostic or therapeutic applications. In addition, chimeric OPG polypeptides may be expressed in vivo and secreted into the circulation to provide therapeutic benefit.

Vectors and Host Cells

Expression vectors containing nucleic acid sequences encoding OPG fusion proteins, host cells transformed with said vectors and methods for the production of OPG fusion proteins are also provided by the invention. An overview of expression of recombinant proteins is found in Methods of Enzymology v. 185, Goeddel, D.V. ed. Academic Press (1990).

Host cells for the production of OPG fusion proteins include procaryotic host cells, such as E. coli, yeast, plant, insect and mammalian host cells.

E. coli strains such as HB101 or JM101 are suitable for expression. Preferred mammalian host cells include COS, CHOd-, 293, CV-1, 3T3, baby hamster kidney (BHK) cells and others. Mammalian host cells are preferred
5 when post-translational modifications, such as glycosylation and polypeptide processing, are important for OPG chimera activity. Mammalian expression allows for the production of secreted polypeptides which may be recovered from the growth medium.

10 Vectors for the expression of OPG chimeric polypeptides contain at a minimum sequences required for vector propagation and for expression of the cloned insert. These sequences include a replication origin, selection marker, promoter, ribosome binding site,
15 enhancer sequences, RNA splice sites and transcription termination site. Vectors suitable for expression in the aforementioned host cells are readily available and the nucleic acids of the invention are inserted into the vectors using standard recombinant DNA techniques.
20 Vectors for tissue-specific expression of OPG chimeric polypeptides are also included. Such vectors include promoters which function specifically in liver, kidney or other organs for production in mice, and viral vectors for the expression of OPG in targeted human
25 cells.

Using an appropriate host-vector system, OPG chimeric polypeptides are produced recombinantly by culturing a host cell transformed with an expression vector containing nucleic acid sequences encoding an
30 OPG chimeric polypeptide under conditions such that the polypeptide is produced, and isolating the product of expression. OPG chimeras are produced in the supernatant of transfected mammalian cells or in inclusion bodies of transformed bacterial host cells.
35 OPG chimeras so produced may be purified by procedures known to one skilled in the art as described below.

Expression vectors for mammalian hosts are exemplified by plasmids such as pDSR α described in PCT Application No. 90/14363; see also Methods in Enzymology vol. 185, D.V. Goeddel, ed. pp. 487-511 for additional examples.

- 5 A variety of expression vectors are available for bacterial host cells and are described in Methods in Enzymology, ibid. pp. 14-37 and references cited therein. It is anticipated that the specific plasmids and host cells described are for illustrative purposes
10 and that the choice of any specific plasmid and host cell for expression of an OPG chimeric polypeptide will depend upon consideration of a variety of factors by one skilled in the art.

15 Antibodies

- Also encompassed by the invention are antibodies specifically binding to an OPG chimeric polypeptide. Antigens for the generation of antibodies may be full-length polypeptides or peptides spanning a
20 portion of the OPG sequence. Immunological procedures for the generation of polyclonal or monoclonal antibodies reactive with OPG are known to one skilled in the art (see, for example, Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor
25 Laboratory Press, Cold Spring Harbor N.Y. (1988)). Antibodies so produced are characterized for binding specificity and epitope recognition using standard enzyme-linked immunosorbent assays. Antibodies also include chimeric antibodies having variable and
30 constant domain regions derived from different species. In one embodiment, the chimeric antibodies are humanized antibodies having murine variable domains and human constant domains. Also encompassed are complementary determining regions grafted to a human
35 framework (so-called CDR-grafted antibodies). Chimeric

and CDR-grafted antibodies are made by recombinant methods known to one skilled in the art. Also encompassed are human antibodies made in mice.

Anti-OPG chimera antibodies of the invention
5 may be used as an affinity reagent to purify OPG from biological samples. In one method, the antibody is immobilized on CNBr-activated Sepharose and a column of antibody-Sepharose conjugate is used to remove OPG from liquid samples. Antibodies are also used as diagnostic
10 reagents to detect and quantitate OPG in biological samples by methods described below.

Pharmaceutical compositions

The invention also provides for
15 pharmaceutical compositions comprising a therapeutically effective amount of an OPG chimeric polypeptide together with a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, preservative and/or adjuvant. The term "therapeutically effective
20 amount" refers to an amount which provides a therapeutic effect for a specified condition and route of administration. The composition may be in a liquid or lyophilized form and comprises a diluent (Tris, acetate or phosphate buffers) having various pH values
25 and ionic strengths, solubilizer such as Tween or Polysorbate, carriers such as human serum albumin or gelatin, preservatives such as thimerosal or benzyl alcohol, and antioxidants such as ascorbic acid or sodium metabisulfite. Also encompassed are
30 compositions comprising OPG chimeric polypeptides modified with water soluble polymers to increase solubility or stability. Compositions may also comprise incorporation of OPG chimeric polypeptides into liposomes, microemulsions, micelles or vesicles
35 for controlled delivery over an extended period of time. Selection of a particular composition will

depend upon a number of factors, including the condition being treated, the route of administration and the pharmacokinetic parameters desired. A more extensive survey of components suitable for pharmaceutical compositions is found in Remington's Pharmaceutical Sciences, 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1980).

Compositions of the invention may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral, nasal, pulmonary or rectal administration. The route of administration eventually chosen will depend upon a number of factors and may be ascertained by one skilled in the art.

Pharmaceutical compositions of chimeric OPG polypeptides are useful for treatment of receptor-mediated disorders, for example disorders resulting from the function (or lack thereof) of protein tyrosine kinases, cytokine, seven transmembrane domain, and cell adhesion receptors. Disorders resulting from the function (or lack thereof) of the corresponding polypeptide ligands of the above referenced receptors may also be treated. In one embodiment, compositions comprising TNF/OPG chimeras are used to treat TNF-related disorders such as inflammation, autoimmune diseases and conditions marked by excessive apoptosis. Chimeras of the invention may act as agonists to stimulate receptor activation and associated changes in cell activity, or chimeras may be antagonists which block receptor function.

The invention also provides for pharmaceutical compositions comprising a therapeutically effective amount of the nucleic acids of the invention together with a pharmaceutically acceptable adjuvant. Nucleic acid compositions will be

suitable for delivery to cells and tissues as part of an anti-sense or gene therapy regimen.

The following examples are offered to more fully illustrate the invention, but are not construed
5 as limiting the scope thereof.

EXAMPLE 1

Construction and Expression of TNFbp/OPG fusion proteins

10

The TNFbp/OPG[196-401] chimeric gene was prepared in a two step PCR process. A first round of PCR was designed to produce overlapping PCR products from each gene. The templates used were plasmids
15 p2302, containing the gene encoding TNFbp 4.0 (Figure 4) fused to the Fc region of human IgG1, and plasmid pRcCMV-human OPG (ATCC accession no. 69969), containing the gene for human OPG. The PCR products were gel purified and used as a template to create the chimeric
20 gene. Primers used for the PCR reactions are as follows: 1275-51 (containing a 5' XbaI site, consensus Kozak and the start of the hTNFbp gene) and 1368-82 (containing a portion of OPG cDNA, an AgeI site and the 3' end of the human TNFbp 4.0 sequence) were used to
25 amplify the TNFbp gene from p2302; 1368-83 (containing the 3' end of TNFbp, an AgeI site and the 5' end of the hOPG C-terminal domain) and 1295-27 (containing a SalI site and the 3' end of the OPG cDNA) were used to amplify the OPG[196-401] gene from pRcCMV-human OPG. A
30 second PCR reaction used primers 1275-51 and 1295-27 to generate the chimeric gene.

The PCR product was cut with XbaI/SalI and subcloned into the pDSR α 2 expression vector to give plasmid p389-1. The expression cassette contains a
35 SV40 early promoter driving the expression of the chimeric gene and also includes an SV40 late intron, an

HTLV translation enhancing signal and an $\alpha 2$ -FSH polyadenylation signal (DeClerck, et al. J. Biol. Chem. 266, 3893-3899 (1991)). The pDSR $\alpha 2$ vector also contains a DHFR cassette for selection in CHO d- cells.

5

Primer Sequences:

1275-51:

(SEQ ID NO:___)

10 5'-CGC TCTAGA CCACC ATG GGC CTC TCC ACC GTG-3'
XbaI Kozak M G L S T V

1368-82:

(SEQ ID NO:___)

15 5'-ACACAGGGTAACATCTAT ACCGGT GGTGCCTGAGTCCTCAG-3'
hOPG C-terminus AgeI hTNFbp

1368-83:

(SEQ ID NO:___)

20 5'-CTGAGGACTCAGGCACC ACCGGT ATAGATGTTACCCTGTG-3'
E D S G T T G I D V T L
TNFbp AgeI hOPG C-terminus

1295-27:

25 (SEQ ID NO:___)

5'-CCTCT GTCGAC TA TTA TAA GCA GCTTATTTTCACGGATTG-3'
Sali * * L C.... OPG-->

Other constructs with truncated OPG
30 dimerization doamins were created as follows:

The primer pair for OPG[194-401] was 1295-27 and 1428-89.

1428-89:

(SEQ ID NO:___)

TCA ACCGGT AAA TGT GGA ATA GAT GTT AC

5 AgeI K C G I D V T

The primer pair for OPG[217-401] was 1295-27 and 1388-50.

10 1388-50:

(SEQ ID NO:___)

GTTT ACCGGT CCT AAC TGG CTT AGT GTC

 AgeI P N W L S V

15 The primer pair for OPG[248-401] was 1295-27 and 1388-51.

1388-51:

(SEQ ID NO:___)

20 AGC ACCGGT GAA CAG ACT TTC CAG CTG

 AgeI E Q T F Q L

The primer pair for OPG[304-401] was 1295-27 and 1388-52.

25

1388-52:

(SEQ ID NO:___)

GGAA ACCGGT CCG GGA AAG AAA GTG GG

 AgeI P G K K V G

30

The corresponding TNFbp/OPG fusion was constructed by excising the AgeI/SalI OPG fragment from p389-1 and replacing it with AgeI/SalI digested OPG PCR products from the above reactions. The amino acid sequences encoded by the above TNFbp/OPG constructs are shown in Figure 4.

35

Transient transfections were performed in COS-7 cells by electroporation. Ten μg of plasmid DNA was electroporated into 2×10^6 cells in 0.8 mls of DMEM. The electroporations were done in 0.4 cm cuvettes at 1.6 kV, 25 mF and 200 ohms. The electroporated cells were plated in 10-cm dishes in DMEM containing 10% FBS, 1x glutamine/penicillin/streptomycin, 1x non-essential amino acids, 1x Na-pyruvate. The following day the media was changed to media containing only 1% FBS. After an additional 72 hours, the conditioned media was harvested and 17 μl was electrophoresed on a 12% denaturing, non-reducing gel. These gels were blotted and analyzed by western blots for the presence of monomer and covalently-linked dimers. The primary antibody was anti-TNFbp (R&D systems, AB-225-PB) at a 1:1000 dilution and the secondary antibody was HRP, rabbit anti-goat (Pierce) at a 1:1000 dilution.

Stable transfections were done in CHO d-cells by calcium phosphate precipitation (DeClerck et al., supra). The transfection was performed as described except that 20 μg of PvuI linearized plasmid was used with 10 μg of herring sperm carrier DNA and 10 μl of calcium phosphate maximizer (Clontech) to transfect to a 10-cm dish containing approximately 5×10^5 cells. After 2 weeks in HT- selection, colonies were ring-cloned and expanded into 24-well plates. Once confluent, two day serum-free conditioned media (SFCM) was prepared and analyzed for the expression of TNFbp/OPG fusion protein by western blot. High expressing clones were expanded and grown in roller bottles for 7d SFCM harvests. The results are shown in Figure 5.

EXAMPLE 2

Biological Activity of TNFbp/OPG chimeric proteins

WEHI Cytotoxicity Assay

5 The WEHI assay is an in vitro cell proliferation assay (Edwards et al. Endocrinology 128,989-996 (1991)). The cell lines are sensitive to TNF- α (i.e., TNF- α is cytotoxic). In the presence of a TNF- α inhibitor, the cells were protected from the cytotoxic effect and thus were able to proliferate.

10 TNF-sensitive WEHI 164 clone 13 cells are suspended at a concentration of 20×10^4 cells/ml in RPMI (Gibco, Grand Island, NY) medium supplemented with 5% Fetal Calf Serum (Hyclone) and penicillin

15 50U/ml:streptomycin 50 mg/ml. One hundred microliters of this cell suspension are placed in each well of flat-bottomed 96-cell microtiter plates, and the cells are allowed to adhere for 4-6 hours at 37°C in 7% CO₂. Medium is then aspirated, and 0.60 mg/ml actinomycin-D

20 (Sigma Chemical Co., St. Louis, MO) is added to each well. A standard curve using serial dilutions at 0, 0.001 0.01, 0.1, 1, 10, 100 U/ml recombinant human TNF is run with each assay. Serially diluted 10-fold concentrations of TNFbp/OPG chimeras from serum-free

25 conditioned medium are further diluted in RPMI-1640 medium containing 5% FBS and then added to duplicate wells (50 μ l/well) containing adherent WEHI 164 cells after the addition of recombinant mouse TNF- α . WEHI-164 clone 13 cells are incubated for 18 hours at 37°C

30 in 5% CO₂. Maximal killing is determined by adding 0.02% Triton X-100 (TX-100) to test wells. After incubation, 70 μ l medium are aspirated, and 50 μ l of a 1 mg/mL solution of the organic dye MTT tetrazolium (3-[4,5-dimethylthiozol-2-yl]2,5-diphenyl tetrazolium bromide; Sigma) is added, and cells are incubated for

35

an additional 4-6 hours. All supernatants are then removed, and 50 μ l DMF/SDS solution (20% SDS, and 50% N,N dimethylformamide, pH 4.7) is added to each well. The DMF/SDS solution is pipetted up and down several
5 times until all MTT crystals are dissolved, and cells were incubated for an additional 2-22 hours. The absorbances (abs) are read on a Vmax reader at 570-650. The percent specific cytotoxicity is calculated from optical densities using the formula: % specific
10 cytotoxicity = $100\% \times [\text{abs}(\text{cells} + \text{medium}) - \text{abs}(\text{cells} + \text{sample})] / [\text{abs}(\text{cells} + \text{medium}) - \text{abs}(\text{cells} + \text{TX-100})]$. The number of units of TNF in each sample is determined using the percent specific cytotoxicities of the murine standards.

15

L929 Cytotoxicity Assay

The L929 cytotoxicity assay is an in vitro cell proliferation assay (Parmely et al. J. Immunol. 151, 389-396 (1993), the disclosure of which is hereby
20 incorporated by reference) which also assesses the cytotoxicity of TNF- α -sensitive killing. The cell lines are sensitive to TNF- α (i.e.; TNF- α is cytotoxic). In the presence of a TNF- α inhibitor, the cells are protected from the cytotoxic effect and thus
25 survive and are able to proliferate.

The L929 cell line was obtained from the American Type Culture Collection (catalog number ATCC CCL 1 NCTC clone 929), as described previously by Parmely et. al. (1993), supra. L929 cells were grown
30 in tissue culture flasks in Dulbecco's MEM with 10 % fetal calf serum (FCS) to 80 % confluence. Cells were trypsinized and seeded at 8,000-10,000 cells/well in 100 ml into Falcon #3072 96 well plates and incubated for 20 to 40 hours at 37 $^{\circ}$ C in 5% CO₂. Samples of
35 TNFbp/Fc or TNFbp/OPG [194-401] polypeptides were

serially diluted in medium and added in triplicate followed by addition of $\text{TNF}\alpha$ to reach a final concentration of 0.5 mg/ml. The cultures were incubated at 37 °C overnight and cell density was measured by
5 crystal violet. Medium was removed by inverting the 96 well plates. Cells were fixed in 100 μl 100% methanol for 2 minutes. After removal of methanol the plates were allowed to dry for 10 minutes. 100 μl of 0.10% crystal violet stain in 20% methanol was added and
10 plates were stained for 10 minutes at room temperature. Excess stain was removed by inverting plates. Plates were washed by dipping three times in ice-cold distilled water and excess water was removed from the wells by gently blotting plates on a tissue.
15 100 μl of 100% methanol was added to stained cells and optical density was measured at 595 nm. Media control reactions contained L929 cells and medium alone, and TNF control reactions contained L929 cells with 0.5 ng/ml $\text{TNF}\alpha$.

20 The activity in this assay of TNFbp/OPG fusions constructed as described in Example 1 is shown in Figure 6.

* * *

25 While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended
30 that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: Amgen Inc.
- (ii) TITLE OF INVENTION: CHIMERIC OPG POLYPEPTIDES
- (iii) NUMBER OF SEQUENCES: 87
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Amgen Inc.
- (B) STREET: 1840 Dehavilland Drive
- (C) CITY: Thousand Oaks
- (D) STATE: California
- (E) COUNTRY: USA
- (F) ZIP: 91320-1789
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Winter, Robert B.
- (C) REFERENCE/DOCKET NUMBER: A-452
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- Asn Ser Ile Cys
1
- (2) INFORMATION FOR SEQ ID NO:2:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
Asn Asn Ser Ile Cys
1 5
- 10 (2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
20
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
25 Gln Asn Asn Ser Ile Cys
1 5
- (2) INFORMATION FOR SEQ ID NO:4:
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
- 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
Pro Gln Asn Asn Ser Ile Cys
1 5
- 45 (2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
50 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
55
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
60 His Pro Gln Asn Asn Ser Ile Cys
1 5

40

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ile His Pro Gln Asn Asn Ser Ile Cys
1 5

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10
- 10 (2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
- 20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
25 1 5 10
- (2) INFORMATION FOR SEQ ID NO:11:
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
- 40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
45 1 5 10
- (2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
50 (A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
- 55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
60 1 5 10 15

42

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile
1 5 10 15
Cys

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
1 5 10 15
Ile Cys

(2) INFORMATION FOR SEQ ID NO:16:

43

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
Phe Cys Cys Ser
1
- (2) INFORMATION FOR SEQ ID NO:17:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:
Phe Cys Cys Ser Leu
1 5
- (2) INFORMATION FOR SEQ ID NO:18:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
Phe Cys Cys Ser Leu Cys
1 5
- (2) INFORMATION FOR SEQ ID NO:19:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 Phe Cys Cys Ser Leu Cys Leu
 1 5

(2) INFORMATION FOR SEQ ID NO:20:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 4 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

 Ala Gln Met Cys
 1

25 (2) INFORMATION FOR SEQ ID NO:21:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
30 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

 Thr Ala Gln Met Cys
 1 5

45 (2) INFORMATION FOR SEQ ID NO:22:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
50 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

 Gln Thr Ala Gln Met Cys
60 1 5

(2) INFORMATION FOR SEQ ID NO:23:

45

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:
Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
Tyr Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
Tyr Tyr Asp Gln Thr Ala Gln Met Cys
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

5 Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
 1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

 Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
 1 5 10

25 (2) INFORMATION FOR SEQ ID NO:28:

 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

40 Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
 1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

60 Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
 1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

47

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
- | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Arg | Leu | Arg | Glu | Tyr | Tyr | Asp | Gln | Thr | Ala | Gln | Met | Cys |
| 1 | | | | 5 | | | | | 10 | | | | |
- (2) INFORMATION FOR SEQ ID NO:31:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 15 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
- | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Cys | Arg | Leu | Arg | Glu | Tyr | Tyr | Asp | Gln | Thr | Ala | Gln | Met | Cys |
| 1 | | | | 5 | | | | | 10 | | | | 15 | |
- (2) INFORMATION FOR SEQ ID NO:32:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 16 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
- | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Thr | Cys | Arg | Leu | Arg | Glu | Tyr | Tyr | Asp | Gln | Thr | Ala | Gln | Met | Cys |
| 1 | | | | 5 | | | | | 10 | | | | | 15 | |
- (2) INFORMATION FOR SEQ ID NO:33:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:
Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met
1 5 10 15
Cys
10
- (2) INFORMATION FOR SEQ ID NO:34:
- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:
Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln
1 5 10 15
Met Cys
30
- (2) INFORMATION FOR SEQ ID NO:35:
- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:
Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala
1 5 10 15
Gln Met Cys
50
- (2) INFORMATION FOR SEQ ID NO:36:
- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr
1 5 10 15
Ala Gln Met Cys
10 20

(2) INFORMATION FOR SEQ ID NO:37:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
1 5 10 15
Thr Ala Gln Met Cys
30 20

(2) INFORMATION FOR SEQ ID NO:38:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp
1 5 10 15
Gln Thr Ala Gln Met Cys
50 20

(2) INFORMATION FOR SEQ ID NO:39:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
60 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
- Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr
1 5 10 15
- Asp Gln Thr Ala Gln Met Cys
20
- 10 (2) INFORMATION FOR SEQ ID NO:40:
- (i) SEQUENCE CHARACTERISTICS:
- 15 (A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
- Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr
1 5 10 15
- 30 Tyr Asp Gln Thr Ala Gln Met Cys
20
- (2) INFORMATION FOR SEQ ID NO:41:
- 35 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
- Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu
1 5 10 15
- 50 Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25
- (2) INFORMATION FOR SEQ ID NO:42:
- 55 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg
1 5 10 15
10 Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:43:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu
1 5 10 15
30 Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:44:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg
1 5 10 15
50 Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25

(2) INFORMATION FOR SEQ ID NO:45:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
- Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys
1 5 10 15
- Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
10 20 25
- (2) INFORMATION FOR SEQ ID NO:46:
- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 20 (ii) MOLECULE TYPE: protein
- 25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:
- Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr
1 5 10 15
- 30 Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25 30
- (2) INFORMATION FOR SEQ ID NO:47:
- 35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 40 (ii) MOLECULE TYPE: protein
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:
- Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
1 5 10 15
- 50 Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys
20 25 30
- (2) INFORMATION FOR SEQ ID NO:48:
- 55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 60 (ii) MOLECULE TYPE: protein

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:
Arg Leu Cys Ala
1

10 (2) INFORMATION FOR SEQ ID NO:49:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:
Arg Leu Cys Ala Pro
25 1 5

(2) INFORMATION FOR SEQ ID NO:50:
(i) SEQUENCE CHARACTERISTICS:
30 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
35 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:
Arg Leu Cys Ala Pro Leu
1 5

45 (2) INFORMATION FOR SEQ ID NO:51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
50 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
Arg Leu Cys Ala Pro Leu Arg
60 1 5

(2) INFORMATION FOR SEQ ID NO:52:

54

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
Arg Leu Cys Ala Pro Leu Arg Lys
1 5
- (2) INFORMATION FOR SEQ ID NO:53:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
Arg Leu Cys Ala Pro Leu Arg Lys Cys
1 5
- (2) INFORMATION FOR SEQ ID NO:54:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
1 5 10
- (2) INFORMATION FOR SEQ ID NO:55:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

55

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 15..32

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

10 CGCTCTAGAC CACC ATG GGC CTC TCC ACC GTG 32
 Met Gly Leu Ser Thr Val
 1 5

(2) INFORMATION FOR SEQ ID NO:56:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

25 Met Gly Leu Ser Thr Val
 1 5

(2) INFORMATION FOR SEQ ID NO:57:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 41 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

ACACAGGGTA ACATCTATAC CGGTGGTGCC TGAGTCCTCA G 41

45 (2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: cDNA

55 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 3..40

60

56

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CT GAG GAC TCA GGC ACC ACC GGT ATA GAT GTT ACC CTG TG 40
Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu
5 1 5 10

(2) INFORMATION FOR SEQ ID NO:59:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

20 Glu Asp Ser Gly Thr Thr Gly Ile Asp Val Thr Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:60:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

CCTCTGTCGA CTATTATAAG CAGCTTATTT TCACGGATTG 40

40 (2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
45 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50 (ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 10..29

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

TCAACCGGT AAA TGT GGA ATA GAT GTT AC 29
Lys Cys Gly Ile Asp Val
1 5
60

57

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Cys Gly Ile Asp Val
1 5

15 (2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

25

(ix) FEATURE:

- (A) NAME/KEY: CDS
(B) LOCATION: 11..28

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GTTTACCGGT CCT AAC TGG CTT AGT GTC
Pro Asn Trp Leu Ser Val
1 5

28

35

(2) INFORMATION FOR SEQ ID NO:64:

40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Pro Asn Trp Leu Ser Val
1 5

50

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:

- 55 (A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 10..27

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

10 AGCACCGGT GAA CAG ACT TTC CAG CTG 27
Glu Gln Thr Phe Gln Leu
1 5

(2) INFORMATION FOR SEO ID NO:66:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

25 Glu Gln Thr Phe Gln Leu
 1 5

(2) INFORMATION FOR SEQ ID NO:67:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 11..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

45 GGAAACCGGT CCG GGA AAG AAA GTG GG 27
Pro Gly Lys Lys Val
1 5

50 (2) INFORMATION FOR SEO ID NO:68:

55 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Pro Gly Lys Lys Val
1 5

59

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

15 Asn Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
 1 5 10 15
 20 Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp
 20 25 30
 Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
 35 40 45
 25 Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
 50 55 60
 Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp
 65 70 75 80
 30 Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Ile Gly His Ala Asn
 85 90 95
 Leu Thr Thr Glu Gln Leu Arg Ile Leu Met Glu Ser Leu Pro Gly Lys
 100 105 110
 Lys Ile Ser Pro Asp Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Pro
 115 120 125
 40 Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
 130 135 140
 Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys
 145 150 155 160
 45 Ala Tyr His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Ile
 165 170 175
 Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe
 180 185 190
 Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 195 200 205

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:

- 60 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

60

(ii) MOLECULE TYPE: protein

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

10 Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
 1 5 10 15
 20 Ala Val Pro Thr Lys Ile Ile Pro Asn Trp Leu Ser Val Leu Val Asp
 20 25 30
 35 Ser Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
 15 35 40 45
 50 Arg Arg His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
 50 55 60
 65 Lys His Gln Asn Arg Asp Gln Glu Met Val Lys Lys Ile Ile Gln Asp
 20 65 70 75 80
 85 Ile Asp Leu Cys Glu Ser Ser Val Gln Arg His Leu Gly His Ser Asn
 25 85 90 95
 100 Leu Thr Thr Glu Gln Leu Leu Ala Leu Met Glu Ser Leu Pro Gly Lys
 100 105 110
 115 Lys Ile Ser Pro Glu Glu Ile Glu Arg Thr Arg Lys Thr Cys Lys Ser
 30 115 120 125
 130 Ser Glu Gln Leu Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
 130 135 140
 145 Asp Gln Asp Thr Leu Lys Gly Leu Met Tyr Ala Leu Lys His Leu Lys
 35 145 150 155 160
 165 Thr Ser His Phe Pro Lys Thr Val Thr His Ser Leu Arg Lys Thr Met
 40 165 170 175
 180 Arg Phe Leu His Ser Phe Thr Met Tyr Arg Leu Tyr Gln Lys Leu Phe
 180 185 190
 195 Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 45 195 200 205

(2) INFORMATION FOR SEQ ID NO:71:

50

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 208 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

60

61

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

5 Lys Cys Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg Phe
 1 5 10 15
 Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val Asp
 20 25 30
 10 Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile Lys
 35 40 45
 Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp
 50 55 60
 15 Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp
 65 70 75 80
 Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn
 85 90 95
 20 Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys
 100 105 110
 25 Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro
 115 120 125
 Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly
 130 135 140
 30 Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys
 145 150 155 160
 Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile
 165 170 175
 35 Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe
 180 185 190
 40 Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 195 200 205

(2) INFORMATION FOR SEQ ID NO:72:

45 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 483 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

55 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..483

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

60 GAT AGT GTG TGT CCC CAA GGA AAA TAT ATC CAC CCT CAA AAT AAT TCG
 Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
 1 5 10 15

48

62

	ATT	TGC	TGT	ACC	AAG	TGC	CAC	AAA	GGA	ACC	TAC	TTG	TAC	AAT	GAC	TGT	96
	Ile	Cys	Cys	Thr	Lys	Cys	His	Lys	Gly	Thr	Tyr	Leu	Tyr	Asn	Asp	Cys	
				20					25					30			
5	CCA	GGC	CCG	GGG	CAG	GAT	ACG	GAC	TGC	AGG	GAG	TGT	GAG	AGC	GGC	TCC	144
	Pro	Gly	Pro	Gly	Gln	Asp	Thr	Asp	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	
			35					40					45				
10	TTC	ACC	GCT	TCA	GAA	AAC	CAC	CTC	AGA	CAC	TGC	CTC	AGC	TGC	TCC	AAA	192
	Phe	Thr	Ala	Ser	Glu	Asn	His	Leu	Arg	His	Cys	Leu	Ser	Cys	Ser	Lys	
		50					55					60					
15	TGC	CGA	AAG	GAA	ATG	GGT	CAG	GTG	GAG	ATC	TCT	TCT	TGC	ACA	GTG	GAC	240
	Cys	Arg	Lys	Glu	Met	Gly	Gln	Val	Glu	Ile	Ser	Ser	Cys	Thr	Val	Asp	
	65					70				75						80	
20	CGG	GAC	ACC	GTG	TGT	GGC	TGC	AGG	AAG	AAC	CAG	TAC	CGG	CAT	TAT	TGG	288
	Arg	Asp	Thr	Val	Cys	Gly	Cys	Arg	Lys	Asn	Gln	Tyr	Arg	His	Tyr	Trp	
					85					90					95		
25	AGT	GAA	AAC	CTT	TTC	CAG	TGC	TTC	AAT	TGC	AGC	CTC	TGC	CTC	AAT	GGG	336
	Ser	Glu	Asn	Leu	Phe	Gln	Cys	Phe	Asn	Cys	Ser	Leu	Cys	Leu	Asn	Gly	
				100					105					110			
30	ACC	GTG	CAC	CTC	TCC	TGC	CAG	GAG	AAA	CAG	AAC	ACC	GTG	TGC	ACC	TGC	384
	Thr	Val	His	Leu	Ser	Cys	Gln	Glu	Lys	Gln	Asn	Thr	Val	Cys	Thr	Cys	
			115					120					125				
35	CAT	GCA	GGT	TTC	TTT	CTA	AGA	GAA	AAC	GAG	TGT	GTC	TCC	TGT	AGT	AAC	432
	His	Ala	Gly	Phe	Phe	Leu	Arg	Glu	Asn	Glu	Cys	Val	Ser	Cys	Ser	Asn	
		130					135					140					
40	TGT	AAG	AAA	AGC	CTG	GAG	TGC	ACG	AAG	TTG	TGC	CTA	CCC	CAG	ATT	GAG	480
	Cys	Lys	Lys	Ser	Leu	Glu	Cys	Thr	Lys	Leu	Cys	Leu	Pro	Gln	Ile	Glu	
	145					150					155					160	
	AAT																483
	Asn																

(2) INFORMATION FOR SEQ ID NO:73:

45	(i)	SEQUENCE CHARACTERISTICS:															
		(A) LENGTH: 161 amino acids															
		(B) TYPE: amino acid															
		(D) TOPOLOGY: linear															
50	(ii)	MOLECULE TYPE: protein															
	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:73:															
55	Asp	Ser	Val	Cys	Pro	Gln	Gly	Lys	Tyr	Ile	His	Pro	Gln	Asn	Asn	Ser	
	1				5					10					15		
	Ile	Cys	Cys	Thr	Lys	Cys	His	Lys	Gly	Thr	Tyr	Leu	Tyr	Asn	Asp	Cys	
				20					25					30			
60	Pro	Gly	Pro	Gly	Gln	Asp	Thr	Asp	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	
			35					40					45				

63

Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
 50 55 60
 5 Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
 65 70 75 80
 Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
 85 90 95
 10 Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
 100 105 110
 Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
 115 120 125
 15 His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
 130 135 140
 20 Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
 145 150 155 160
 Asn

25 (2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 705 base pairs
 (B) TYPE: nucleic acid
 30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

35

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..705

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

45	TTG CCC GCC CAG GTG GCA TTT ACA CCC TAC GCC CCG GAG CCC GGG AGC Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser	48
	1 5 10 15	
50	ACA TGC CGG CTC AGA GAA TAC TAT GAC CAG ACA GCT CAG ATG TGC TGC Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys	96
	20 25 30	
55	AGC AAG TGC TCG CCG GGC CAA CAT GCA AAA GTC TTC TGT ACC AAG ACC Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr	144
	35 40 45	
60	TCG GAC ACC GTG TGT GAC TCC TGT GAG GAC AGC ACA TAC ACC CAG CTC Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu	192
	50 55 60	
65	TGG AAC TGG GTT CCC GAG TGC TTG AGC TGT GGC TCC CGC TGT AGC TCT Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser	240
	65 70 75 80	

64

	GAC CAG GTG GAA ACT CAA GCC TGC ACT CGG GAA CAG AAC CGC ATC TGC	288
	Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys	
	85 90 95	
5	ACC TGC AGG CCC GGC TGG TAC TGC GCG CTG AGC AAG CAG GAG GGG TGC	336
	Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys	
	100 105 110	
10	CGG CTG TGC GCG CCG CTG CGC AAG TGC CGC CCG GGC TTC GGC GTG GCC	384
	Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala	
	115 120 125	
15	AGA CCA GGA ACT GAA ACA TCA GAC GTG GTG TGC AAG CCC TGT GCC CCG	432
	Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro	
	130 135 140	
20	GGG ACG TTC TCC AAC ACG ACT TCA TCC ACG GAT ATT TGC AGG CCC CAC	480
	Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His	
	145 150 155 160	
	CAG ATC TGT AAC GTG GTG GCC ATC CCT GGG AAT GCA AGC AGG GAT GCA	528
	Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala	
	165 170 175	
25	GTC TGC ACG TCC ACG TCC CCC ACC CGG AGT ATG GCC CCA GGG GCA GTA	576
	Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val	
	180 185 190	
30	CAC TTA CCC CAG CCA GTG TCC ACA CGA TCC CAA CAC ACG CAG CCA ACT	624
	His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr	
	195 200 205	
35	CCA GAA CCC AGC ACT GCT CCA AGC ACC TCC TTC CTG CTC CCA ATG GGC	672
	Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly	
	210 215 220	
40	CCC AGC CCC CCA GCT GAA GGG AGC ACT GGC GAC	705
	Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp	
	225 230 235	

(2) INFORMATION FOR SEQ ID NO:75:

45	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 235 amino acids
	(B) TYPE: amino acid
	(D) TOPOLOGY: linear
50	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:
55	Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr Ala Pro Glu Pro Gly Ser
	1 5 10 15
	Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys
	20 25 30
60	Ser Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr
	35 40 45
	Ser Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu
	50 55 60

65

Trp Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser
 65 70 75 80
 5 Asp Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys
 85 90 95
 Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys
 100 105 110
 10 Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala
 115 120 125
 Arg Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro
 130 135 140
 Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His
 145 150 155 160
 20 Gln Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Arg Asp Ala
 165 170 175
 Val Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val
 180 185 190
 25 His Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr
 195 200 205
 Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly
 210 215 220
 Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp
 225 230 235
 35 (2) INFORMATION FOR SEQ ID NO:76:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 420 amino acids
 (B) TYPE: amino acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 45
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:
 50 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 25 30
 55 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80

	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	Phe	Thr	Ala	Ser	Glu	Asn	His	Leu
					85					90					95	
5	Arg	His	Cys	Leu	Ser	Cys	Ser	Lys	Cys	Arg	Lys	Glu	Met	Gly	Gln	Val
				100					105					110		
	Glu	Ile	Ser	Ser	Cys	Thr	Val	Asp	Arg	Asp	Thr	Val	Cys	Gly	Cys	Arg
			115					120					125			
10	Lys	Asn	Gln	Tyr	Arg	His	Tyr	Trp	Ser	Glu	Asn	Leu	Phe	Gln	Cys	Phe
		130					135					140				
	Asn	Cys	Ser	Leu	Cys	Leu	Asn	Gly	Thr	Val	His	Leu	Ser	Cys	Gln	Glu
15	145					150					155					160
	Lys	Gln	Asn	Thr	Val	Cys	Thr	Cys	His	Ala	Gly	Phe	Phe	Leu	Arg	Glu
					165					170					175	
20	Asn	Glu	Cys	Val	Ser	Cys	Ser	Asn	Cys	Lys	Lys	Ser	Leu	Glu	Cys	Thr
				180					185					190		
	Lys	Leu	Cys	Leu	Pro	Gln	Ile	Glu	Asn	Val	Lys	Gly	Thr	Glu	Asp	Ser
			195					200					205			
25	Gly	Thr	Thr	Gly	Lys	Cys	Gly	Ile	Asp	Val	Thr	Leu	Cys	Glu	Glu	Ala
		210					215					220				
	Phe	Phe	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser
30	225					230					235					240
	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val
					245					250					255	
35	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu
				260					265					270		
	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys
			275					280					285			
40	Ile	Ile	Gln	Asp	Ile	Asp	Leu	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile
		290					295					300				
	Gly	His	Ala	Asn	Leu	Thr	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser
45	305					310					315					320
	Leu	Pro	Gly	Lys	Lys	Val	Gly	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys
					325					330					335	
50	Ala	Cys	Lys	Pro	Ser	Asp	Gln	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
				340					345					350		
	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	His	Ala	Leu
			355					360					365			
55	Lys	His	Ser	Lys	Thr	Tyr	His	Phe	Pro	Lys	Thr	Val	Thr	Gln	Ser	Leu
		370					375					380				
	Lys	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe	Thr	Met	Tyr	Lys	Leu	Tyr
60	385					390					395					400
	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys
					405					410					415	

Ile Ser Cys Leu
420

5 (2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 211 amino acids
(B) TYPE: amino acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

20 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30
25 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45
30 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80
35 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110
40 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125
45 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160
50 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190
55 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205
60 Gly Thr Thr
210

68

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 417 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

15 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 20 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 20 25 30
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 25 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80
 30 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
 85 90 95
 35 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
 100 105 110
 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
 115 120 125
 40 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
 130 135 140
 Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
 145 150 155 160
 45 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
 165 170 175
 50 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
 180 185 190
 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
 195 200 205
 55 Gly Thr Thr Gly Ile Asp Val Thr Leu Cys Glu Glu Ala Phe Phe Arg
 210 215 220
 Phe Ala Val Pro Thr Lys Phe Thr Pro Asn Trp Leu Ser Val Leu Val
 225 230 235 240
 60 Asp Asn Leu Pro Gly Thr Lys Val Asn Ala Glu Ser Val Glu Arg Ile
 245 250 255

69

Lys Arg Gln His Ser Ser Gln Glu Gln Thr Phe Gln Leu Leu Lys Leu
 260 265 270
 5 Trp Lys His Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln
 275 280 285
 Asp Ile Asp Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala
 290 295 300
 10 Asn Leu Thr Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly
 305 310 315 320
 Lys Lys Val Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys
 325 330 335
 15 Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn
 340 345 350
 20 Gly Asp Gln Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser
 355 360 365
 Lys Thr Tyr His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr
 370 375 380
 25 Ile Arg Phe Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu
 385 390 395 400
 Phe Leu Glu Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys
 405 410 415
 30 Leu

(2) INFORMATION FOR SEQ ID NO:79:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 397 amino acids
 (B) TYPE: amino acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

50 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 25 30
 55 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 60 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80

70

	Cys	Arg	Glu	Cys	Glu	Ser	Gly	Ser	Phe	Thr	Ala	Ser	Glu	Asn	His	Leu	
					85					90					95		
5	Arg	His	Cys	Leu	Ser	Cys	Ser	Lys	Cys	Arg	Lys	Glu	Met	Gly	Gln	Val	
				100					105					110			
	Glu	Ile	Ser	Ser	Cys	Thr	Val	Asp	Arg	Asp	Thr	Val	Cys	Gly	Cys	Arg	
			115					120					125				
10	Lys	Asn	Gln	Tyr	Arg	His	Tyr	Trp	Ser	Glu	Asn	Leu	Phe	Gln	Cys	Phe	
		130					135					140					
	Asn	Cys	Ser	Leu	Cys	Leu	Asn	Gly	Thr	Val	His	Leu	Ser	Cys	Gln	Glu	
15						150					155					160	
	Lys	Gln	Asn	Thr	Val	Cys	Thr	Cys	His	Ala	Gly	Phe	Phe	Leu	Arg	Glu	
					165					170					175		
20	Asn	Glu	Cys	Val	Ser	Cys	Ser	Asn	Cys	Lys	Lys	Ser	Leu	Glu	Cys	Thr	
				180					185					190			
	Lys	Leu	Cys	Leu	Pro	Gln	Ile	Glu	Asn	Val	Lys	Gly	Thr	Glu	Asp	Ser	
			195					200					205				
25	Gly	Thr	Thr	Gly	Pro	Asn	Trp	Leu	Ser	Val	Leu	Val	Asp	Asn	Leu	Pro	
		210					215					220					
	Gly	Thr	Lys	Val	Asn	Ala	Glu	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	
30		225				230				235						240	
	Ser	Ser	Gln	Glu	Gln	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	
					245					250					255		
35	Asn	Lys	Asp	Gln	Asp	Ile	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	
				260					265					270			
	Cys	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr	Phe	
			275					280					285				
40	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys	Lys	Val	Gly	
		290					295					300					
	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys	Ala	Cys	Lys	Pro	Ser	Asp	Gln	
45		305				310					315					320	
	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg	Ile	Lys	Asn	Gly	Asp	Gln	Asp	
				325						330					335		
50	Thr	Leu	Lys	Gly	Leu	Met	His	Ala	Leu	Lys	His	Ser	Lys	Thr	Tyr	His	
				340					345						350		
	Phe	Pro	Lys	Thr	Val	Thr	Gln	Ser	Leu	Lys	Lys	Thr	Ile	Arg	Phe	Leu	
			355					360					365				
55	His	Ser	Phe	Thr	Met	Tyr	Lys	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	
		370					375					380					
	Ile	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	Cys	Leu				
60		385				390					395						

71

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 366 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

15 Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 20 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 20 25 30
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 25 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80
 30 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
 85 90 95
 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
 100 105 110
 35 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
 115 120 125
 40 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
 130 135 140
 Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
 145 150 155 160
 45 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
 165 170 175
 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
 180 185 190
 50 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
 195 200 205
 55 Gly Thr Thr Gly Glu Gln Thr Phe Gln Leu Leu Lys Leu Trp Lys His
 210 215 220
 Gln Asn Lys Asp Gln Asp Ile Val Lys Lys Ile Ile Gln Asp Ile Asp
 225 230 235 240
 60 Leu Cys Glu Asn Ser Val Gln Arg His Ile Gly His Ala Asn Leu Thr
 245 250 255

72

Phe Glu Gln Leu Arg Ser Leu Met Glu Ser Leu Pro Gly Lys Lys Val
 260 265 270
 5 Gly Ala Glu Asp Ile Glu Lys Thr Ile Lys Ala Cys Lys Pro Ser Asp
 275 280 285
 Gln Ile Leu Lys Leu Leu Ser Leu Trp Arg Ile Lys Asn Gly Asp Gln
 290 295 300
 10 Asp Thr Leu Lys Gly Leu Met His Ala Leu Lys His Ser Lys Thr Tyr
 305 310 315 320
 His Phe Pro Lys Thr Val Thr Gln Ser Leu Lys Lys Thr Ile Arg Phe
 325 330 335
 15 Leu His Ser Phe Thr Met Tyr Lys Leu Tyr Gln Lys Leu Phe Leu Glu
 340 345 350
 20 Met Ile Gly Asn Gln Val Gln Ser Val Lys Ile Ser Cys Leu
 355 360 365

(2) INFORMATION FOR SEQ ID NO:81:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 311 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1 5 10 15
 40 Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
 20 25 30
 His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
 35 40 45
 45 Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
 50 55 60
 50 Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65 70 75 80
 Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
 85 90 95
 55 Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
 100 105 110
 Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
 115 120 125
 60 Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
 130 135 140

73

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
 145 150 155 160
 5 Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
 165 170 175
 Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
 180 185 190
 10 Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
 195 200 205
 Gly Thr Thr Gly Pro Gly Lys Lys Val Gly Ala Glu Asp Ile Glu Lys
 210 215 220
 15 Thr Ile Lys Ala Cys Lys Pro Ser Asp Gln Ile Leu Lys Leu Leu Ser
 225 230 235 240
 Leu Trp Arg Ile Lys Asn Gly Asp Gln Asp Thr Leu Lys Gly Leu Met
 245 250 255
 His Ala Leu Lys His Ser Lys Thr Tyr His Phe Pro Lys Thr Val Thr
 260 265 270
 25 Gln Ser Leu Lys Lys Thr Ile Arg Phe Leu His Ser Phe Thr Met Tyr
 275 280 285
 Lys Leu Tyr Gln Lys Leu Phe Leu Glu Met Ile Gly Asn Gln Val Gln
 290 295 300
 30 Ser Val Lys Ile Ser Cys Leu
 305 310

(2) INFORMATION FOR SEQ ID NO:82:

35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 106 amino acids
 (B) TYPE: amino acid
 40 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

50 Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
 1 5 10 15
 Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
 20 25 30
 55 Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
 35 40 45
 Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
 50 55 60
 60 Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
 65 70 75 80

74

Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
85 90 95

5 Trp Ser Glu Asn Leu Phe Gln Cys Phe Cys
100 105

(2) INFORMATION FOR SEQ ID NO:83:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
1 5 10 15
25 Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
20 25 30
Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
35 40 45
30 Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
50 55 60
Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
65 70 75 80
Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
85 90 95
40 Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
100 105

(2) INFORMATION FOR SEQ ID NO:84:

45 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: protein

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Met Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn
1 5 10 15
60 Ser Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp
20 25 30

75

Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly
 35 40 45
 5 Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser
 50 55 60
 Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val
 65 70 75 80
 10 Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr
 85 90 95
 Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
 100 105
 15

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

30 Met Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His
 1 5 10 15
 35 Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr
 20 25 30
 Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His
 35 40 45
 40 Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln
 50 55 60
 45 Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys
 65 70 75 80
 Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys
 85 90 95
 50 Phe Asn Cys Ser Leu
 100

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 91 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

5 Met Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro
 1 5 10 15
 Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe
 20 25 30
 10 Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys
 35 40 45
 Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg
 50 55 60
 15 Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser
 65 70 75 80
 20 Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
 85 90

(2) INFORMATION FOR SEQ ID NO:87:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 94 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: protein

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Met Ser Ile Ser Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn
 1 5 10 15
 40 Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser
 20 25 30
 Gly Ser Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys
 35 40 45
 45 Ser Lys Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr
 50 55 60
 50 Val Asp Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His
 65 70 75 80
 Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu
 85 90

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising an amino acid
5 sequence of an osteoprotegerin dimerization domain
fused to a heterologous amino acid sequence.
2. The polypeptide of Claim 1 wherein the
heterologous amino acid sequence and the
10 osteoprotegerin dimerization domain are human.
3. The polypeptide of Claim 1 wherein the
heterologous amino acid sequence and the
osteoprotegerin dimerization domain are from different
15 species.
4. The polypeptide of Claim 1 covalently
associated with one or more chimeric polypeptides which
result in a multimeric polypeptide complex.
20
5. The polypeptide of Claim 4 wherein the complex
is a dimer.
6. The polypeptide of Claim 1 wherein the
25 heterologous amino acid sequence is a membrane-bound
receptor lacking functional membrane associated amino
acid sequences.
7. The polypeptide of Claim 6 wherein the receptor
30 is selected from the group consisting of receptor
tryrosine kinases, cytokine receptors, seven
transmembrane domain receptors, and cell adhesion
receptors.

8. The polypeptide of Claim 1 wherein the heterologous amino acid sequence is selected from members of the tumor necrosis factor-like receptor family consisting of TNFR-1, TNFR-2, TNFRp, NGFR, FasB, CD40, OX40, CD27, CD30, and 4-1BB.

9. The polypeptide of Claim 8 wherein the heterologous sequence comprises TNFR-1 lacking functional membrane-associated sequences.

10. The polypeptide of Claim 9 wherein the heterologous sequence is a 30 kDa TNF inhibitor, a 40 kDa TNF inhibitor, or an analog thereof.

11. The polypeptide of Claim 1 wherein the carboxy terminus of the heterologous sequence is fused to the amino terminus of the OPG dimerization domain.

12. The polypeptide of Claim 1 wherein the amino terminus of the heterologous sequence is fused to the carboxy terminus of the OPG dimerization domain.

13. The polypeptide of Claim 1 wherein one or more amino acids are inserted between the heterologous sequence and the OPG dimerization domain.

14. A multimeric polypeptide comprising covalently associated monomers of OPG chimeric polypeptides.

15. The multimeric polypeptide of Claim 14 which is a dimer.

16. An isolated nucleic acid sequence encoding the polypeptide of Claim 1.

17. An expression vector comprising the nucleic acid sequence of Claim 16.

5 18. A host cell transformed or transfected with the expression vector of Claim 17 in a manner allowing expression of the nucleic acid.

10 19. A pharmaceutical composition comprising the polypeptide of any of Claims 1 to 15.

FIGURE 1

Rat:	Asn	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala		
Phe Phe														
Mouse:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala	Phe	Phe
Human:	Lys	<u>Cys</u>	Gly	Ile	Asp	Val	Thr	Leu	<u>Cys</u>	Glu	Glu	Ala	Phe	Phe
Rat:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Ile	Ile	Pro	Asn	Trp	Leu	Ser
Mouse:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Ile	Ile	Pro	Asn	Trp	Leu	Ser
Human:	Arg	Phe	Ala	Val	Pro	Thr	Lys	Phe	Thr	Pro	Asn	Trp	Leu	Ser
Rat:	Val	Leu	Val	Asp	Ser	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Mouse:	Val	Leu	Val	Asp	Ser	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Human:	Val	Leu	Val	Asp	Asn	Leu	Pro	Gly	Thr	Lys	Val	Asn	Ala	Glu
Rat:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Arg	His	Ser	Ser	Gln	Glu	Gln
Mouse:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Arg	His	Ser	Ser	Gln	Glu	Gln
Human:	Ser	Val	Glu	Arg	Ile	Lys	Arg	Gln	His	Ser	Ser	Gln	Glu	Gln
Rat:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Arg	Asp
Mouse:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Arg	Asp
Human:	Thr	Phe	Gln	Leu	Leu	Lys	Leu	Trp	Lys	His	Gln	Asn	Lys	Asp
Rat:	Gln	Glu	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Mouse:	Gln	Glu	Met	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Human:	Gln	Asp	Ile	Val	Lys	Lys	Ile	Ile	Gln	Asp	Ile	Asp	Leu	<u>Cys</u>
Rat:	Glu	Ser	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr
Mouse:	Glu	Ser	Ser	Val	Gln	Arg	His	Leu	Gly	His	Ser	Asn	Leu	Thr
Human:	Glu	Asn	Ser	Val	Gln	Arg	His	Ile	Gly	His	Ala	Asn	Leu	Thr
Rat:	Thr	Glu	Gln	Leu	Arg	Ile	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Mouse:	Thr	Glu	Gln	Leu	Leu	Ala	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Human:	Phe	Glu	Gln	Leu	Arg	Ser	Leu	Met	Glu	Ser	Leu	Pro	Gly	Lys
Rat:	Lys	Ile	Ser	Pro	Asp	Glu	Ile	Glu	Arg	Thr	Arg	Lys	Thr	<u>Cys</u>
Mouse:	Lys	Ile	Ser	Pro	Glu	Glu	Ile	Glu	Arg	Thr	Arg	Lys	Thr	<u>Cys</u>
Human:	Lys	Val	Gly	Ala	Glu	Asp	Ile	Glu	Lys	Thr	Ile	Lys	Ala	<u>Cys</u>
Rat:	Lys	Pro	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
Mouse:	Lys	Ser	Ser	Glu	Gln	Leu	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg
Human:	Lys	Pro	Ser	Asp	Gln	Ile	Leu	Lys	Leu	Leu	Ser	Leu	Trp	Arg

FIGURE 1 (Con't)

Rat:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	Tyr
Mouse:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	Tyr
Human:	Ile	Lys	Asn	Gly	Asp	Gln	Asp	Thr	Leu	Lys	Gly	Leu	Met	His

Rat:	Ala	Leu	Lys	His	Leu	Lys	Ala	Tyr	His	Phe	Pro	Lys	Thr	Val
Mouse:	Ala	Leu	Lys	His	Leu	Lys	Thr	Ser	His	Phe	Pro	Lys	Thr	Val
Human:	Ala	Leu	Lys	His	Ser	Lys	Thr	Tyr	His	Phe	Pro	Lys	Thr	Val

Rat:	Thr	His	Ser	Leu	Arg	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe
Mouse:	Thr	His	Ser	Leu	Arg	Lys	Thr	Met	Arg	Phe	Leu	His	Ser	Phe
Human:	Thr	Gln	Ser	Leu	Lys	Lys	Thr	Ile	Arg	Phe	Leu	His	Ser	Phe

Rat:	Thr	Met	Tyr	Arg	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile
Mouse:	Thr	Met	Tyr	Arg	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile
Human:	Thr	Met	Tyr	Lys	Leu	Tyr	Gln	Lys	Leu	Phe	Leu	Glu	Met	Ile

Rat:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu
Mouse:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu
Human:	Gly	Asn	Gln	Val	Gln	Ser	Val	Lys	Ile	Ser	<u>Cys</u>	Leu

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FIGURE 2

30kDa TNF Inhibitor

5' -GATAGTGTGTGTCCCCAAGGAAAATATATCCACCCTCAAAATAATTGATTGTGCTGTACC-
+-----+-----+-----+-----+-----+-----+-----+-----+
D S V C P Q G K Y I H P Q N N S I C C T -
-AAGTGCCACAAAGGAACCTACTTGTACAATGACTGTCCAGGCCCGGGGCAGGATACGGAC-
+-----+-----+-----+-----+-----+-----+-----+-----+
K C H K G T Y L Y N D C P G P G Q D T D -
-TGCAGGGAGTGTGAGAGCGGCTCCTTCACCGCTTCAGAAAACCACCTCAGACACTGCCTC-
+-----+-----+-----+-----+-----+-----+-----+-----+
C R E C E S G S F T A S E N H L R H C L -
-AGCTGCTCCAAATGCCGAAAGGAAATGGGTGAGGTGGAGATCTCTTCTTGACAGTGGAC-
+-----+-----+-----+-----+-----+-----+-----+-----+
S C S K C R K E M G Q V E I S S C T V D -
-CGGGACACCGTGTGTGGCTGCAGGAAGAACCAGTACCGGCATTATTGGAGTGAAAACCTT-
+-----+-----+-----+-----+-----+-----+-----+-----+
R D T V C G C R K N Q Y R H Y W S E N L -
-TTCCAGTGCTTCAATTGCAGCCTCTGCCTCAATGGGACCGTGCACCTCTCCTGCCAGGAG-
+-----+-----+-----+-----+-----+-----+-----+-----+
F Q C F N C S L C L N G T V H L S C Q E -
-AAACAGAACACCGTGTGCACCTGCCATGCAGTTTCTTTCTAAGAGAAAACGAGTGTGTC-
+-----+-----+-----+-----+-----+-----+-----+-----+
K Q N T V C T C H A G F F L R E N E C V -
-TCCTGTAGTAACTGTAAGAAAAGCCTGGAGTGCACGAAGTTGTGCCTACCCCAGATTGAG-
+-----+-----+-----+-----+-----+-----+-----+-----+
S C S N C K K S L E C T K L C L P Q I E -
-AAT-3'
+-----+
N *

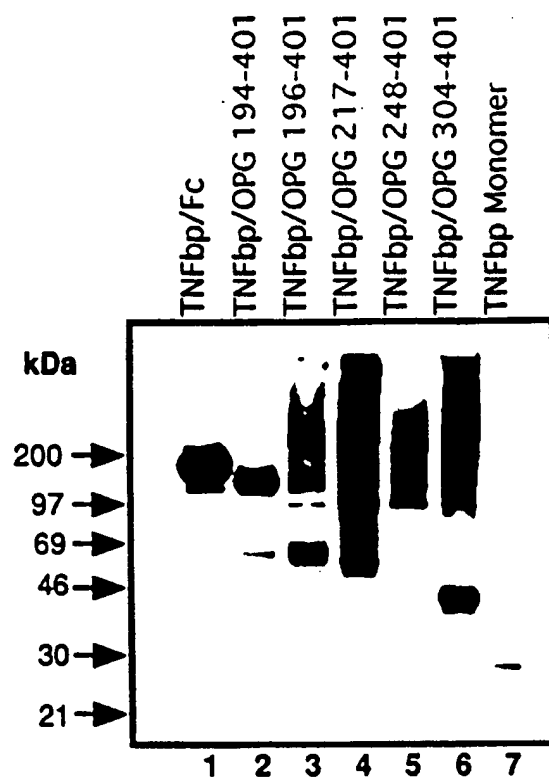
FIGURE 4

	1					50
TNFbp/OPG	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
TNFbp 4.0	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
TNFbp/196	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
TNFbp/217	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
TNFbp/248	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
TNFbp/304	<u>MGLSTVPDLL LPLVLLELLV GIYPSGVIGL VPHLGDREKR</u>					DSVCPQGKYI
	51					100
TNFbp/OPG	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
TNFbp 4.0	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
TNFbp/196	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
TNFbp/217	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
TNFbp/248	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
TNFbp/304	HPQNNISICCT KCHKGTLYLN DCPGPGQDTD CRECESGSFT					ASENHLRHCL
	101					150
TNFbp/OPG	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
TNFbp 4.0	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
TNFbp/196	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
TNFbp/217	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
TNFbp/248	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
TNFbp/304	SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSEN					FQCFNCSLCL
	151					200
TNFbp/OPG	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
TNFbp 4.0	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
TNFbp/196	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
TNFbp/217	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
TNFbp/248	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
TNFbp/304	NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE					CTKLCLPQIE
	201					250
TNFbp/OPG	NVKGTEDESGT TGKCGIDVTL CEEAFFRFAV PTKFTPNWLS					VLVDNLPGT
TNFbp 4.0	NVKGTEDESGT T.....				
TNFbp/196	NVKGTEDESGT T...GIDVTL CEEAFFRFAV PTKFTPNWLS					VLVDNLPGT
TNFbp/217	NVKGTEDESGT TG.....				PNWLS VLVDNLPGT
TNFbp/248	NVKGTEDESGT TG.....				
TNFbp/304	NVKGTEDESGT TG.....				
		196 (OPG)				217 (OPG)
	251					300
TNFbp/OPG	VNAESVERIK RQHSSQEQTFF QLLKLWKHQ					NDQDIVKKII QDIDLCENS
TNFbp 4.0
TNFbp/196	VNAESVERIK RQHSSQEQTFF QLLKLWKHQ					NDQDIVKKII QDIDLCENS
TNFbp/217	VNAESVERIK RQHSSQEQTFF QLLKLWKHQ					NDQDIVKKII QDIDLCENS
TNFbp/248EQTF QLLKLWKHQ					NDQDIVKKII QDIDLCENS
TNFbp/304
		248 (OPG)				

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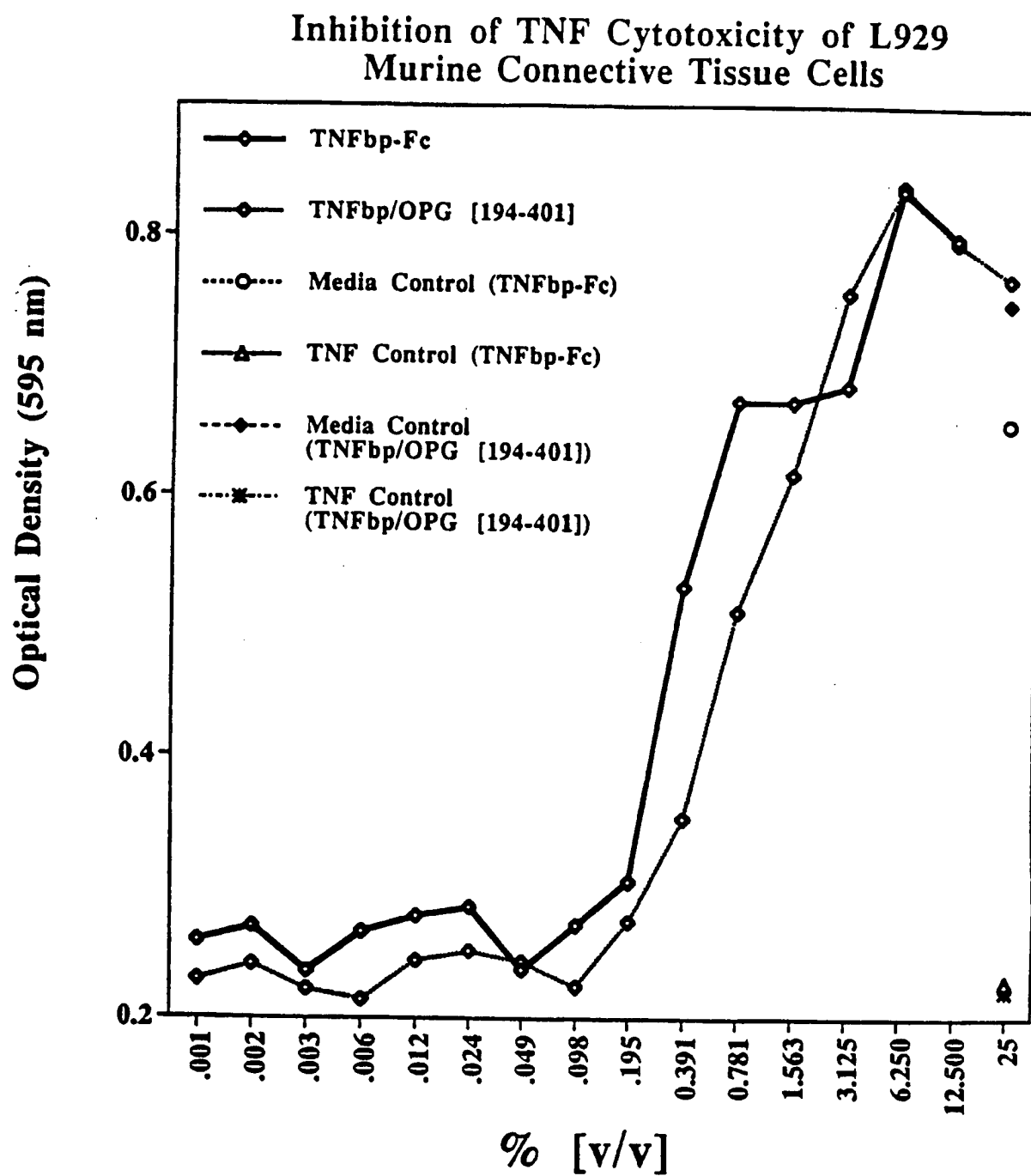
	301				350
TNFbp/OPG	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp 4.0
TNFbp/196	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/217	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/248	QRHIGHANLT	FEQLRSLMES	LPGKKVGAED	IEKTIKACKP	SDQILKLLSL
TNFbp/304PGKKVGAED	IEKTIKACKP	SDQILKLLSL
			304 (OPG)		
	351				400
TNFbp/OPG	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp 4.0
TNFbp/196	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/217	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/248	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
TNFbp/304	WRIKNGDQDT	LKGLMHALKH	SKTYHFPKTV	TQSLKKTIRF	LHSFTMYKLY
	401		420		
TNFbp/OPG	QKL FLEMIGN	QVQSVKISCL			
TNFbp 4.0			
TNFbp/196	QKL FLEMIGN	QVQSVKISCL			
TNFbp/217	QKL FLEMIGN	QVQSVKISCL			
TNFbp/248	QKL FLEMIGN	QVQSVKISCL			
TNFbp/304	QKL FLEMIGN	QVQSVKISCL			
			401 (OPG)		

FIGURE 5



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FIGURE 6



INTERNATIONAL SEARCH REPORT

Internatl Application No

PCT/US 98/08631

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/62 C07K14/705 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search

10 September 1998

Date of mailing of the International search report

25/09/1998

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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